

HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF ETHANOL ROOT EXTRACT OF *CLERODENDRUM PHLOMIDIS* IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: In traditional systems of medicine numerous plants have been used for the treatment and control of diabetes. *Clerodendrum phlomidis* is the accepted botanical source of the ayurvedic drug *Agnimantha*. The plant is used in the treatment of smallpox, inflammation, coryza, scrotal enlargement, syphilitic, and postnatal complaints. The roots are used to treat measles, gonorrhoea, and diabetes. The present study aims at evaluating the hypoglycemic and hypolipidemic activity of *C. phlomidis* roots.

Methods: Diabetes was induced by administering 120 mg/kg alloxan monohydrate intraperitoneally in albino Wistar rats for a treatment period of 21 days during which body weight changes and fasting blood glucose (FBG) were monitored at weekly intervals. On the 21st-day serum biochemical parameters, liver malondialdehyde, reduced glutathione, and glycogen levels were estimated.

Results: The ethanol extract at 400 mg/kg dose significantly ($p < 0.001$) reduced the elevated FBG and serum biochemical parameter levels.

Conclusion: The present study substantiates the traditional use of *C. phlomidis* in the treatment of diabetes.

Keywords: Alloxan, Antidiabetic activity, *Clerodendrum phlomidis*, Hypolipidemic, Liver glycogen.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting a large number of people in the world. Nearly 285 million people of the world population were known to be affected by diabetes in the year 2010, and this number may increase up to 439 million by 2030 [1]. This metabolic disorder occurs due to defects in insulin secretion and action. As the insulin secretion is hampered, it directly affects the action of insulin on glucose metabolism resulting in loss of homeostasis of glucose in the body. This also causes impaired metabolism of fats, carbohydrates, and proteins [2]. Diabetes mellitus is also characterized by glucosuria, ketonemia, hyperglycemia, and negative nitrogen balance which is caused due to deficiency of insulin [3].

Diabetes mellitus can be treated by improving the availability of insulin in the body and also by overcoming the resistance of insulin. To improve the insulin available to the body, drugs underclass of sulfonylurea, and meglitinide are used. Drugs belonging to biguanides, thiazolidinediones, and α -glucosidase inhibitors are used to overcome insulin resistance. Apart from these commercially available synthetic drugs diabetes can also be treated with herbal drugs. There is a great demand for a new ayurvedic based drug for diabetes in the developed and developing countries because of their wide biological activities and higher safety margin than the synthetic drugs. In Ayurveda, plants such as *Phyllanthus amarus*, *Gymnema sylvestre*, *Trigonella foenum*, *Terminalia chebula*, and *Momordica charantia* [4] are used in the management of diabetes mellitus.

Agnimantha is one of the important drugs in Ayurveda [5]. The accepted botanical source of Agnimantha is *C. phlomidis* L.f. belonging to the family Verbenaceae [6]. The plant is used in the treatment of smallpox, inflammation, coryza, scrotal enlargement, syphilitic, and postnatal complaints [7-9]. Roots are used in the treatment of measles, gonorrhoea, diabetes, and as a bitter tonic [5]. Some species of *Clerodendrum* such as *Clerodendrum serratum* (leaves) [10],

Clerodendrum viscosum (leaves) [11], and *Clerodendrum inerme* (aerial parts) [12] are reported for antidiabetic activity. *C. phlomidis* is traditionally used in the treatment of diabetes [13], but significant hypoglycemic and other pharmacological activities are reported on the leaves although root being the part of the plant used (crude drug). In a clinical trial, the hypoglycemic activity of *C. phlomidis* leaf was found to be effective compared to tolbutamide [13]. Since no work has been reported on the antidiabetic activity of *C. phlomidis* roots, the present study has been undertaken.

METHODS

Chemicals

Alloxan monohydrate was procured from Avra Synthesis Pvt. Ltd., Hyderabad, India, and all the reagent kits were obtained from Robonik Pvt. Ltd., Navi Mumbai, Maharashtra, India, and Diatek, Kolkata, India.

Plant material

The root of *C. phlomidis* was collected from Thoothukudi district of Tamil Nadu, India in January 2017. The plant was identified and authenticated by Dr. V. Chelladurai, Taxonomist. Herbarium of the plant material was prepared [14] and deposited in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India (Voucher specimen No. 052).

Preparation of ethanol extract

Total ethanol extract was prepared by extracting powdered crude drug with 95% ethanol by continuous hot percolation using Soxhlet apparatus. The extract was filtered and concentrated to dryness under reduced temperature and pressure. Preliminary phytochemical screening was performed for the detection of primary and secondary metabolites [15,16].

Determination of total phenolic and flavonoid content

The total phenolic content was determined by Folin-Ciocalteu method using gallic acid as standard [17] and total flavonoid content was

estimated by aluminum chloride colorimetric assay using Quercetin as a standard drug [18].

Animals

Healthy Albino Wistar rats of either sex weighing 200–300 g were used. The animals were housed in the Animal House of Department of Pharmacology, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India. All the animals had free access to food and water *ad libitum* and were kept under laboratory condition (12 h day and 12 h night cycle at $21 \pm 2^\circ\text{C}$). All diseased rats and those outside the weight range were excluded from the study. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC No.: XVIII/MSRFP/M-04/08.02.2017).

Acute toxicity study

Acute toxicity of ethanol root extract was determined as per the OECD guidelines 423. Limit test was performed by administering a dose of 2000 mg/kg of ethanol extract to a group of 3 female animals. Animal behavior and toxicity signs were observed for 15 days and further until the end of the study [19].

Induction of diabetes

Diabetes was induced in the experimental animals by administering a single dose of 120 mg/kg body weight of alloxan monohydrate intraperitoneally. After 7 days, animals with fasting blood glucose (FBG) level of more than 200 mg/dL were considered as diabetic and used for the study [20-22].

Experimental protocol

Nondiabetic rats served as normal group animals, and the diabetic animals were grouped into 4 groups of 6 animals each.

Group	Treatment
I	Normal control (2% acacia)
II	Positive control (alloxan 120 mg/kg)
III	Standard drug (glibenclamide 0.5 mg/kg)
IV	Ethanol extract test dose 200 mg/kg
V	Ethanol extract test dose 400 mg/kg

On 0, 7th, 14th, and 21st day, body weight changes and FBG levels of animals in all groups were monitored.

Estimation of biochemical parameters

On the 21st day, animals were fasted overnight, anesthetized with ether anesthesia and blood was withdrawn from retro-orbital plexus. Serum was separated from the blood by centrifugation at 10,000 rpm for 10 min (Micro Centrifuge, REMI Motors Ltd, Mumbai) for the estimation of various biochemical parameters such as glycosylated hemoglobin (HbA1c), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and total protein using reagent kits. Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels in the serum were derived using Friedewald's formula:

Low-density lipoprotein (LDL) concentration (mg/dL) = Total cholesterol - (HDL cholesterol + Triglycerides/5)

Very low-density lipoprotein (VLDL) concentration (mg/dL) = Triglycerides/5

Further, the animals were sacrificed with thiopental sodium, and the liver was isolated for the estimation of malondialdehyde (MDA), reduced glutathione and glycogen levels [23,24].

Histopathological studies

All the animals were sacrificed on the 21st day, and necropsy was carried out. The pancreases were collected, washed with normal saline and kept in 10% formalin solution. Paraffin sections were made at 5 mm thickness using a microtome processed in alcohol-xylene series. It was stained with eosin and hematoxylin, mounted on the glass slide for histopathological evaluation.

Oral glucose tolerance test (OGTT)

OGTT was performed in normal rats (Albino Wistar strain) of either sex weighing from 200 to 300 g. The animals were divided into 5 groups consisting of 6 animals each in a group. The animals were fasted overnight before the study. During the treatment, Group I served as control and received 2% acacia solution (p.o.). Group II served as positive control and did not receive any treatment before administration of 2 % glucose solution (p.o.). Group III served as standard and received 0.5 mg/kg dose of glibenclamide (p.o.). Groups IV and V received low and high (200 and 400 mg/kg) doses of ethanol extract (p.o.). After 60 min of standard and extract administration, all animals were given 2 g/kg body weight of glucose (p.o.). Immediately blood samples were collected at every 0, 30, 60, 90, and 120 min from the tail vein and blood glucose levels were checked using an ACE Blood Glucometer [25].

Statistical analysis

The data were expressed as a mean \pm standard error of the mean values and tested with one-way analysis of variance followed by Tukey-Kramer multiple comparison tests.

Table 1: Phytochemical screening of *C. phlomidis* ethanol root extract

Test for	Ethanol extract
Alkaloids	+
Carbohydrates	+
Phytosterols	+
Fixed oil and fats	-
Phenolic compounds and tannins	+
Glycosides	-
Saponins	-
Flavonoids	+
Proteins and amino acids	-

+: Present, -: Absent, *C. phlomidis*: *Clerodendrum phlomidis*

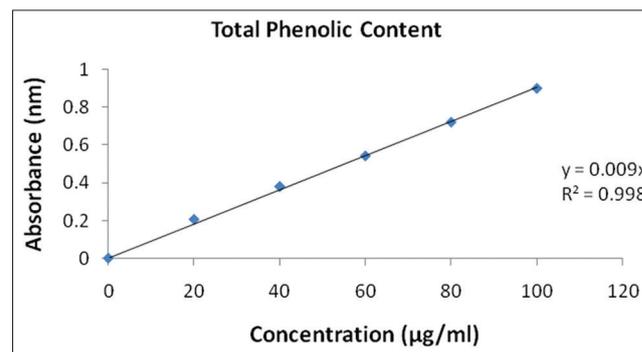


Fig. 1: Calibration curve of standard gallic acid

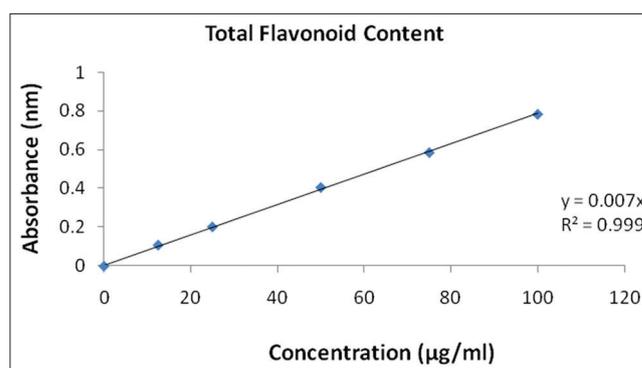


Fig. 2: Calibration curve of standard quercetin

RESULTS

Phytochemical studies

The preliminary phytochemical screening of the ethanol extract of *C. phlomidis* root was shown in Table 1.

Total phenolic and flavonoid content

The calibration curve of gallic acid and quercetin was derived and presented in Figs. 1 and 2. The phenolic and flavonoid content of ethanol root extract was shown in Table 2.

Acute toxicity study

The acute toxicity study of ethanol extract did not show any toxic symptoms or death at 2000 mg/kg dose for up to 15 days. Hence, 1/5th and 1/10th of the maximum dose tested were selected for the evaluation of the antidiabetic activity.

Antidiabetic activity

Effect on body weight and FBG level

The normal and standard treated group animals showed a weight gain of 1.67 and 9.5 g, respectively. The extract treated animals at a dose of

200 and 400 mg/kg showed a significant increase in body weight of 5.66 and 8.5 g, respectively, whereas positive control group showed a weight loss of 17.17 g (Table 3).

On the 0 day, the FBG level of all the experimental animals was found to increase after administration of 120 mg/kg dose of alloxan monohydrate (i.p.). The animals treated with Glibenclamide 0.5 mg/kg showed extremely significant ($p < 0.001$) decrease in FBG level when compared to positive control on the 21st day of the study. At 400 mg/kg dose, ethanol root extract showed significant ($p < 0.001$) decrease in FBG level compared to positive control (Table 4).

Effect on serum biochemical parameters

Lipid profile

Administration of alloxan at a dose of 120 mg/kg caused a significant ($p < 0.001$) increase in TC, TG levels, and reduction in HDL levels compared to the control group. The diabetic animals treated with glibenclamide 0.5 mg/kg showed extremely significant ($p < 0.001$) decrease in TC and TG levels in comparison to the positive control group. Animals treated with *C. phlomidis* ethanol root extract at high dose level (400 mg/kg) showed extremely significant ($p < 0.001$) decrease in TC and TG levels (Table 5).

HbA1c

There was a significant ($p < 0.001$) elevation in HbA1c level in positive group animals when compared to control group. Animals treated with ethanol extract at both dose levels (200 and 400 mg/kg) showed extremely significant ($p < 0.001$) decrease in elevated HbA1c level in comparison to positive control group (Table 5).

Table 2: Total phenolic and flavonoid content of ethanol root extract of *C. phlomidis*

Extract	Total phenolic content GAE (mg/g)	Total flavonoid content (mg/g)
Ethanol extract	89.22	61.17

C. phlomidis: *Clerodendrum phlomidis*, GAE: Gallic acid equivalent

Table 3: Effect of ethanol root extract of *C. phlomidis* on body weight

Group	Treatment	Mean initial body weight (g)	Mean final body weight (g)	Mean weight gain/weight loss (g)
I	Normal	255.83±25.82	257.5±25.77	1.67↑
II	Positive control	287±22.36	269.83±21.45	17.17↓
III	Glibenclamide (0.5 mg/kg)	287.5±22.63	297±24.66	9.5↑
IV	Ethanol extract (200 mg/kg)	260±13.33	265.66±17.03	5.66↑
V	Ethanol extract (400 mg/kg)	293.66±25.81	302.16±27.57	8.5↑

C. phlomidis: *Clerodendrum phlomidis*

Table 4: Effect of *C. phlomidis* on fasting blood glucose in alloxan-induced diabetic rats

Group	Treatment	FBG (mg/dl)			
		0 day	7 th day	14 th day	21 st day
I	Normal	89.84±5.54***	90.82±5.25***	91.01±5.22***	91.50±5.12***
II	Positive control	213.80±3.48 ^a	214.65±4.38 ^a	215.39±4.04 ^a	216.65±2.58 ^a
III	Glibenclamide (0.5 mg/kg)	202.88±2.85***	187.83±3.30***	160.74±7.65***	123.76±3.52***
IV	Ethanol extract (200 mg/kg)	206.84±3.16 ^{ns}	203.64±2.65 ^{ns}	199.49±1.67 ^{ns}	187.77±3.89*
V	Ethanol extract (400 mg/kg)	207.67±2.80 ^{ns}	201.77±2.08 ^{ns}	189.91±1.32**	144.15±6.13***

Values are expressed as mean±SEM and statistically analyzed by one-way ANOVA followed by Turkey Kramer multiple comparison tests. *** $p < 0.001$, ** $p < 0.01$, * $p > 0.05$, ^{ns} $p > 0.05$ in comparison with positive control. ^a $p < 0.001$ in comparison with normal control. *C. phlomidis*: *Clerodendrum phlomidis*, FBG: Fasting blood glucose, SEM: Standard error of mean

Table 5: Effect of ethanol root extract of *C. phlomidis* on serum biochemical parameters

Group	Treatment	TP (mg/dl)	HbA1c (%)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Normal	9.11±0.13***	5.58±0.56***	139.22±1.76***	72.23±1.24***	84.03±2.73***	40.74±2.01***	14.11±0.25***
II	Positive control	4.06±0.82 ^a	9.27±0.56 ^a	187.97±2.53 ^a	168.04±4.00 ^a	48.74±3.73 ^a	104.98±4.17 ^a	33.60±0.79 ^a
III	Glibenclamide (0.5 mg/kg)	9.06±0.22***	6.12±0.08***	143.94±2.14***	78.53±3.38***	80.05±2.73***	48.18±1.94***	15.70±0.66***
IV	Ethanol extract (200 mg/kg)	4.60±0.25 ^{ns}	7.2±0.04***	170.44±4.63**	110.12±6.06***	64.03±1.34*	84.53±3.31**	22.02±1.21***
V	Ethanol extract (400 mg/kg)	8.21±0.19***	6.31±0.05***	146.30±1.60***	81.07±1.81***	86.59±0.55***	43.48±1.78***	16.10±0.45***

Values are expressed as mean±SEM and statistically analyzed by one-way ANOVA followed by Turkey Kramer multiple comparison tests. *** $p < 0.001$, ** $p < 0.01$, * $p > 0.05$, ^{ns} $p > 0.05$ in comparison with positive control. ^a $p < 0.001$ in comparison with control. *C. phlomidis*: *Clerodendrum phlomidis*, TP: Total protein, HbA1c: Glycosylated hemoglobin, TC: Total cholesterol, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, SEM: Standard error of the mean

Effect on liver antioxidants levels

The positive control group showed significant ($p < 0.001$) increase in MDA and decrease in reduced glutathione levels when compared to control group. Administration of ethanol root extract of *C. phlomidis* for 21 days at both the dose levels showed extremely significant ($p < 0.001$) decrease in MDA level and increase in reduced glutathione level, and the values were comparable with standard glibenclamide (0.5 mg/kg) (Table 6).

Liver glycogen

There was an extremely significant ($p < 0.001$) decrease in liver glycogen levels of positive control animals when compared to vehicle-treated group. Treatment of glibenclamide at a dose of 0.5 mg/kg showed extremely significant ($p < 0.001$) increase in liver glycogen level in comparison to the positive control group. Treatment with ethanol extract at dose of 400 mg/kg showed significant ($p > 0.05$) increase in liver glycogen level in comparison to positive control (Table 6).

OGTT

Administration of glucose 2 g/kg caused a significant ($p < 0.001$) elevation in blood glucose level of the animals at a different time interval (0, 30, 60, 90, and 120 min) when compared to the positive control group. Treatment with glibenclamide (0.5 mg/kg) significantly ($p < 0.01$; $p < 0.001$) lowered the blood glucose level at different time interval in comparison to positive control. Groups treated with ethanol extract at low- and high-dose level (200 and 400 mg/kg), respectively, showed significant ($p > 0.05$; $p < 0.01$; $p < 0.001$) decrease in blood glucose level at different time intervals when compared to positive control group. The ethanol extract was found to be effective to stimulate the pancreatic β cells to secrete insulin after an overdose of glucose maintaining a normal blood glucose level (Table 7).

Histopathological studies

The pancreas of control group animals showed lobular cells surrounded by islets of Langerhans. The acinar cells are surrounded with intact interlobular connective tissue and polygonal cells are arranged normally. The pancreas of positive control animals showed congested dilated blood vessels and degeneration of islet cells. Glibenclamide (standard) treated group pancreas showed regeneration of islets cells surrounded by acinar cells. The pancreas of animals treated with ethanol extract (200 and 400 mg/kg) showed regeneration of islet cells (Fig. 3a-e).

DISCUSSION

Preliminary phytochemical screening of ethanol extract showed the presence of alkaloids, carbohydrates, phytosterols, and phenolic compounds such as flavonoids and tannins. The ethanol extract was found to contain 89.22 mg/g of phenolic and 61.17 mg/g of flavonoid content. Acute toxicity study revealed that the extract did not show any toxic symptoms at a dose of 2000 mg/kg. Hence, 1/5th and 1/10th of the maximum dose administered were selected for the antidiabetic study.

Antidiabetic activity of the root extract of *C. phlomidis* was evaluated by alloxan-induced diabetic model. The diabetogenic action of alloxan is mainly due to the formation of reactive oxygen species as a result of the reduction of alloxan to diuretic acid in the pancreas or due to its potential to react with protein SH group of the pancreatic β cells causing cell death. The free radicals formed causes cell death by damaging the DNA of the β cells leading to induction of Type II diabetes [26]. During the study, body weights of the animals were monitored at weekly intervals, and it was found that the extract treated groups showed a significant increase in body weight when compared to standard. In diabetic condition, the body either does not produce insulin or fails to secrete or use insulin effectively leading to insulin deficiency which causes rapid gluconeogenesis. Administration of ethanol extract of *C. Phlomidis* at a higher dose (400 mg/kg) for 21 days showed a significant decrease in FBG level when compared to positive control.

Insulin is responsible for lipid metabolism as it inhibits the process of lipolysis. In diabetic condition, the action of insulin is altered due to the deficiency of insulin causing the release of free fatty acids which undergo β -oxidation resulting in increase in TC levels. Insulin deficiency inhibits the metabolism of LDL due to a decrease in 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity. The decrease in enzyme activity leads to an increase in LDL concentration giving rise to lipid deposition. Lack of insulin also leads to deposition of TGs due to inactivation of the enzyme protein lipase responsible for hydrolysis of TGs. Hence, in diabetic condition, there is an increase in TC, LDL, TG levels, and decrease in HDL level due to metabolic abnormalities [2,21]. The elevated TC, TGs, and LDL and VLDL levels in diabetes-induced animals were significant decrease in the group treated with 400 mg/kg ethanol root extract of *C. phlomidis* when compared to the positive control animal. The HDL levels were also restored to the normal levels in the extract treated group.

Table 6: Effect of *C. phlomidis* on liver antioxidants and glycogen levels

Group	Treatment	MDA (nmoles/100 mg)	Reduced glutathione (nmoles/100 mg)	Glycogen (mg/100 mg)
I	Normal	0.203±0.03***	71.18±1.81***	2.88±0.28***
II	Positive control	0.88±0.06 ^a	17.55±1.40 ^a	0.81±0.22 ^a
III	Glibenclamide (0.5 mg/kg)	0.337±0.02***	66.37±0.83***	2.61±0.24***
IV	Ethanol extract (200 mg/kg)	0.529±0.03***	55.86±3.93***	1.73±0.09 ^{ns}
V	Ethanol extract (400 mg/kg)	0.324±0.02***	65.30±1.80***	2.04±0.12*

Values are expressed as mean±SEM and statistically analyzed by one-way ANOVA followed by Turkey Kramer multiple comparison tests. *** $p < 0.001$, ** $p < 0.01$, * $p > 0.05$, ^{ns} $p > 0.05$ in comparison with positive control. ^a $p < 0.001$ in comparison with control. SEM: Standard error of the mean, *C. phlomidis*: *Clerodendrum phlomidis*, MDA: Malondialdehyde

Table 7: Effect of *C. phlomidis* on OGTT in normal rats

Group	Treatment	Blood glucose level (mg/dl)				
		0 min	30 min	60 min	90 min	120 min
I	Normal	46.33±1.11***	64.5±4.08***	92±1.98***	86.33±1.90***	65.03±4.67***
II	Positive control (2 g/kg glucose)	89.83±4.43 ^a	122.83±2.28 ^a	128.66±1.99 ^a	118±2.03 ^a	91.16±2.27 ^a
III	Glibenclamide (0.5 mg/kg)	85.16±2.35 ^{ns}	117.16±2.34**	127.83±2.58**	114.16±2.02***	88±3.60 ^{ns}
IV	Ethanol extract (200 mg/kg)	71.5±3.18*	78.3±3.14***	80.66±2.90***	79.33±2.92***	75.83±2.57 ^{ns}
V	Ethanol extract (400 mg/kg)	74.16±3.32 ^{ns}	110±4.76 ^{ns}	135.83±8.36 ^{ns}	117.16±8.49**	84.5±5.01 ^{ns}

Values are expressed as mean±SEM and statistical analysed by one-way ANOVA followed by Turkey Kramer multiple comparison tests. *** $p < 0.001$, ** $p < 0.01$, * $p > 0.05$, ^{ns} $p > 0.05$ in comparison with positive control. ^a $p < 0.001$ in comparison with control. SEM: Standard error of the mean, *C. phlomidis*: *Clerodendrum phlomidis*, OGTT: Oral glucose tolerance test

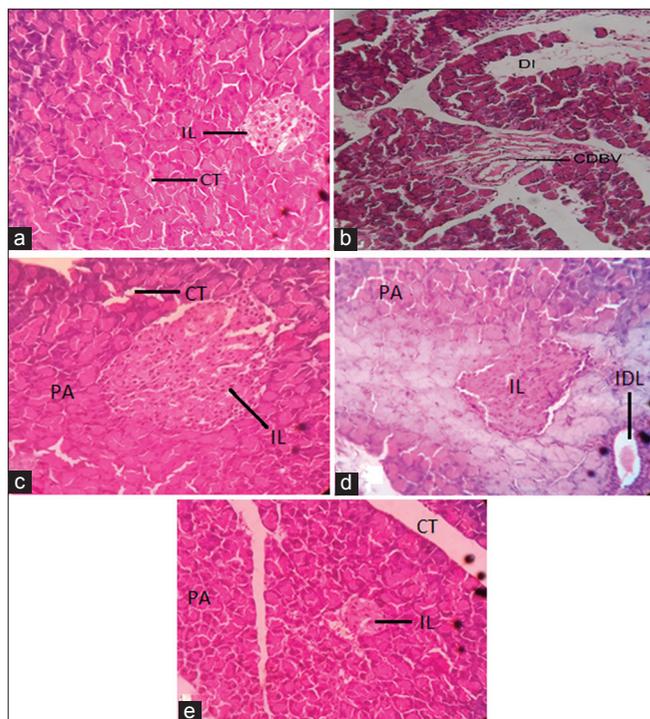


Fig. 3: Photomicrographs of a section of pancreas showing histological changes. (a) Normal control, (b) diabetic rats, (c) diabetic rats treated with Glibenclamide, (d) diabetic rats treated with ethanol extract of *C. phlomidis* (200 mg/kg), and (e) diabetic rats treated with ethanol extract of *Clerodendrum phlomidis* (400 mg/kg). IL: Islets of Langerhans, PA: Pancreatic cells, CT: Connective tissue, ILD: Interlobular duct, CDBV: Congested dilated blood vessels, DI: Destroyed islets (blank spots)

HbA1c test helps to determine the average blood glucose levels for the preceding 3 months. In diabetic condition, high levels of HbA1c are observed due to the non-enzymatic reaction between glucose present in the plasma and the β chain. The glucose molecule circulating in the blood binds to hemoglobin giving rise to hyperglycemic condition [27]. There was a significant decrease in the HbA1c levels in the group treated with *C. phlomidis* ethanol root extract at 400 mg/kg dose in comparison to the positive control.

One of the complications of diabetes mellitus is liver damage. Insulin resistance may lead to liver damage causing cirrhosis and liver failure. MDA is a by-product of lipid peroxidation which helps to detect tissue damage caused by the free radical reaction. In diabetic condition, the concentration of MDA is decreased due to the free radical formation by lipid peroxidation. This causes an increase in oxidative stress as a result of high levels of peroxides [28]. MDA levels were restored to normal range in the extract treated group at a higher dose of 400 mg/kg and comparable with the standard glibenclamide. Reduced glutathione is one of the antioxidant enzymes which protect the liver cells from oxidative damage. In diabetic condition, the reduced glutathione levels are decreased due to increase in oxidative stress caused on the liver [26]. The level of reduced glutathione was increased significantly in the animals treated with ethanol root extract of *C. phlomidis* at both the doses (200 and 400 mg/kg) when compared to positive control.

Liver also plays a significant role in the regulation of carbohydrate metabolism. Glycogen synthesis is impaired in a diabetic condition causing accumulation of glycogen in the liver. This occurs due to the dysfunction of liver glycogen synthase which leads to abnormalities in liver enzyme activity [29]. The group treated with *C. phlomidis* ethanol root extract at a dose of 400 mg/kg showed an increase in the liver glycogen level when compared to positive control. The glucose

tolerance test is done to determine the body's capability to use glucose. This test helps to detect pre-diabetic and diabetic condition [25]. The group treated with ethanol extract at a dose of 200 mg/kg showed a decrease in blood glucose level between 30 and 90 min whereas extract dose of 400 mg/kg significantly decreases the blood glucose level only at 90 min. It was found that the root extract of *C. phlomidis* has the potential to lower the blood glucose level after induction of hyperglycemia by an overload of glucose.

The antidiabetic activity of *C. phlomidis* leaf has been reported by Gopinathan and Naveenraj [30]. In their study, a marked decrease in the levels of biochemical parameters such as blood glucose, liver glycogen, and HbA1c was observed which was comparable with the standard drug. The findings of the present study correlate with the reported antidiabetic activity of *C. Phlomidis* leaf which substantiates the traditional use of *C. phlomidis* in the treatment of diabetes.

Several studies have correlated the antidiabetic activity of the medicinal plants with the presence of phenolic compounds [31,32]. The preliminary phytochemical screening showed the presence of flavonoids which may be responsible for the antidiabetic activity. The extract also showed the presence of alkaloids in the preliminary organic analysis which have also been reported to possess antidiabetic effect [33,34]. Hence, these phytoconstituents may be responsible for the antidiabetic activity of *C. Phlomidis* root.

CONCLUSION

The present study confirms the antidiabetic activity of ethanol root extract of *C. phlomidis*. The study also substantiates the traditional use of *C. phlomidis* in the treatment of *prameha* (diabetes mellitus) in Ayurveda. Further isolation of active principle responsible for the hypoglycemic activity has to be undertaken which will provide a lead molecule for the treatment of diabetes.

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AUTHORS' CONTRIBUTIONS

Mrunal Shanbhag, Postgraduate student in the Department of Pharmacognosy, performed the experimental work and manuscript writing. Gowri Radhakrishnan, Assistant Professor, Department of Pharmacognosy designed, guided the experimental work and edited the manuscript.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest.

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